

APPLICANTS: **Blackburn et al.**
U.S.S.N.: **10/532,834**

Listing of the Claims:

This listing of claims will replace all prior versions and listing of claims in the application:

1-39. (Cancelled)

40. (Currently Amended) A method of detecting protein expression and folding comprising providing a cellular lysate comprising a protein fused to a ble marker protein; contacting a surface derivatized with ~~antibiotic from~~ a bleomycin family antibiotic with said lysate; and assessing binding of said fusion protein to said ~~immobilized~~ antibiotic, wherein binding of said fusion protein to said antibiotic indicates expression and folding of said protein.

41. (Previously presented) The method of claim 40, wherein said ble marker protein is an expression and folding marker.

42. (Previously Presented) The method of claim 40, wherein said ble marker protein is an affinity tag.

43. (Cancelled)

44. (Currently amended) The method of claim 40, wherein said ble marker protein is the expression product of a Sh ble gene, Tn 5 gene, or Sa ble gene.

45. (Withdrawn) A method of immobilizing a protein to a surface, comprising providing the protein to the surface as a ble fusion protein and wherein the surface is a surface derivatized with an antibiotic from the bleomycin family.

46. (Withdrawn) The method of claim 45, wherein the antibiotic from the bleomycin family is selected from the group consisting of bleomycin, phleomycin, tallysomycin, pepleomycin and Zeocin™.

47. (Withdrawn) The method of claim 45 wherein the antibiotic from the bleomycin family is selected from the group consisting of bleomycin A2, bleomycin A5, bleomycin A6, bleomycin B2 or Zeocin™.

48. (Withdrawn) The method of claim 45, wherein a functional group on the antibiotic is used to link it to the surface.

49. (Withdrawn) The method of claim 48, wherein an amine group present on the antibiotic is used to link it to the surface.

50. (Withdrawn) The method of claim 45, wherein the surface is the surface of an array, a microtiter plate, a slide or a bead.

51. (Withdrawn) The method of claim 45, wherein the surface is the surface of an array, a microtiter plate, a slide or a bead.

52. (Withdrawn) The method of claim 51, wherein the array is a microarray.

53. (Withdrawn) The method of claim 52, wherein the array is a MALDI array.

54. (Withdrawn) The method of claim 51, further comprising removing the ble fusion protein from the surface.

55. (Withdrawn) A probe comprising a target surface comprising an array having a plurality of discrete target areas presenting one or more analyte capture moieties comprising an antibiotic from the bleomycin family.

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56. (Withdrawn) The probe of claim 55, wherein the antibiotic is provided on the target surface at a high surface density.

57. (Withdrawn) The probe of claim 56, wherein the capture moieties have an affinity for the moiety they are intended to capture in the order of 100 nM.

58. (Withdrawn) The probe of claim 55, wherein the antibiotic from the bleomycin family is selected from the group consisting of bleomycin, phleomycin, tallysomycin, pepleomycin and ZeocinTM.

59. (Withdrawn) The probe of claim 55, wherein the antibiotic from the bleomycin family is selected from the group consisting of bleomycin A2, bleomycin A5, bleomycin A6, bleomycin B2 and ZeocinTM.

60. (Withdrawn) A purification media comprising a large surface to volume area comprising a target surface presenting one or more analyte capture moieties comprising an antibiotic from the bleomycin family.

61. (Withdrawn) The purification media of claim 60 which is a bead.

62. (Withdrawn) The purification media of claim 60, wherein the antibiotic is provided on the target surface at a low surface density.

63. (Withdrawn) The purification media of claim 62, wherein the capture moieties have affinity for the moiety they are intended to capture in the order of 600 nM.

64. (Withdrawn) The purification media of claim 60, wherein the antibiotic from the bleomycin family is selected from the group consisting of bleomycin, phleomycin, tallysomycin, pepleomycin and ZeocinTM.

65. (Withdrawn) The purification media of claim 60, wherein the antibiotic from the bleomycin family is selected from the group consisting of bleomycin A2, bleomycin A5, bleomycin A6, bleomycin B2 and ZeocinTM.

66. (Withdrawn) The purification media of claim 60, wherein the antibiotic is bound to the surface via a flexible linker molecule.

67. (Withdrawn) The purification media of claim 66, wherein the flexible linker molecule is a polyethylene glycol (PEG).

68. (Withdrawn) A method for generating soluble forms of an insoluble protein comprising the steps of :

- i) generating a library of protein variants; and
- ii) selecting colonies for the presence of a soluble protein by expressing the protein as a ble fusion protein and selecting an antibiotic from the bleomycin family.

69. (Withdrawn) The method of claim 68 further comprising the steps of growing the selected colonies, lysing them and binding the fusion protein to a surface.

70. (Withdrawn) The method of claim 69 wherein the surface comprises an antibiotic form the bleomycin family via which the fusion protein is bound.

71. (Currently amended) A method of claim 40 further comprising purifying a ble fusion protein from a crude extract comprising immobilizing the ble fusion protein on a surface derivatized with antibiotic from the bleomycin family and *optionally* releasing it therefrom.

72. (Withdrawn) A method of identifying the cellular localization of a protein comprising the steps of:

- i) expressing the protein as a ble fusion protein in a cell;
- ii) introducing a labeled antibiotic from the bleomycin family into the cell; and

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iii) detecting the labeled antibiotic.

73. (Withdrawn) The method of claim 72, wherein the antibiotic is an antibiotic from the bleomycin family characterized in that it is tagged with a marker.

74. (Withdrawn) The method of claim 73, wherein the marker is a visual marker.

75. (Withdrawn) The method of claim 74, wherein the visual marker is a fluorescent marker.

76. (Withdrawn) The method of claim 75, wherein the fluorescent marker is selected from NHS-activated fluorescein, Cy3, Cy5, or Rhodamine.

77. (Withdrawn) A kit for the production of an array comprising a ble vector and a surface derivatized with an antibiotic from the bleomycin family or the components for making said derivatized surface.

78. (Cancelled)

79. (Previously presented) The method of claim 40 wherein the antibiotic binding of the ble fusion protein is determined by mass spectrometry.

80. (Currently amended) The method of claim 40 wherein the antibiotic binding of the ble fusion protein is detected by labeling the antibiotic with a marker and detecting binding of the ble fusion protein to the labeled antibiotic by detecting said marker.

81. (Cancelled)